The CTC-Chip

An Exciting New Tool to Detect Circulating Tumor Cells in Lung Cancer Patients

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Abstract: Circulating tumor cells (CTCs) are rare cells that originate from a malignancy and circulate freely in the peripheral blood. The ability to capture and study CTCs is an emerging field with implications for early detection, diagnosis, determining prognosis and monitoring of cancer, as well as for understanding the fundamental biology of the process of metastasis. Here, we review the development and initial clinical studies with a novel microfluidic platform for isolating these cells, the CTC-chip, and discuss its potential uses in the study of lung cancer.

Key Words: Circulating tumor cells, Non-small cell lung cancer, EGFR mutation, CTC-chip.

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For one hundred and forty years, we have known that there are tumor cells circulating in the peripheral blood of patients with cancer.[1] The modern understanding of cancer biology and the process of metastasis suggests that these circulating cells play a vital role in forming metastatic disease.[2] However, studying this cellular population has been particularly challenging because their extreme rarity in comparison to hematologic cells (about 1 tumor cell per 1 billion blood cells) has made them difficult to isolate. Over the past decade, progress has been made in the field through the introduction of several new technologies, most notably an epithelial antibody-coated magnetic bead isolation system that has allowed gross quantification of circulating tumor cells (CTCs) from the blood of cancer patients and has been useful in prognostication in patients with advanced breast, colon, and prostate cancers.[3–6] Here, we will summarize the findings with a recently described microfluidic-based technology called the CTC-chip, that may make it possible to expand CTC detection from a prognostic indicator to a detection and surveillance tool as well as an invaluable research adjunct.[7,8]

The CTC-chip technology is unique because its use of microfluidics provides a platform by which one can vastly increase the sensitivity and yield of capturing rare cell populations from whole blood, while doing so in a gentle manner that preserves the viability of isolated CTCs. Microfluidics is an emerging multidisciplinary field in which engineering, physics, chemistry, microtechnology, and biotechnology intersect to create controlled nano-scale environments in which biologic assays can be performed that are not possible at the macro-scale.[9] The ability to perform such novel microassays arises from the exploitation of differences in fluid dynamic properties that result from manipulation of fluid across microscopic distances in a rapid time frame at the nano-scale compared with the macro-scale. Furthermore, using microfluidic devices, researchers are able to create better controlled microenvironments to manipulate cells, and transport reagents.

The CTC-chip itself is a silicon chip the size of a standard microscope slide on which an array of 78,000 μm-sized posts are etched with a specific geometric pattern and then are coated, or "functionalized," with antibodies to epithelial cell adhesion molecule (EpCAM). Whole blood from patients is pneumatically pushed over the surface of the CTC-chip and through the forest of microposts, Figure 1. The fluid dynamics imposed by the geometric arrangement of the posts leads the cellular component of the blood down specific streamlines that are interjected frequently by the posts, thereby maximizing interaction of the CTCs with the EpCAM-functionalized surfaces, and resulting in high-efficiency capture of the CTCs directly onto the sides of the posts. The captured cells can then be confirmed as CTCs (through staining which differentiates nonspecifically bound leukocytes from epithelial CTCs), counted, and further analyzed in a variety of ways including molecular characterization. Because the CTC-chip employs whole blood without any preprocessing and the sheer stress experienced by CTCs as they travel through the chip is minimal, 98% of captured cells remain viable.[7] The platform is flexible, in that different

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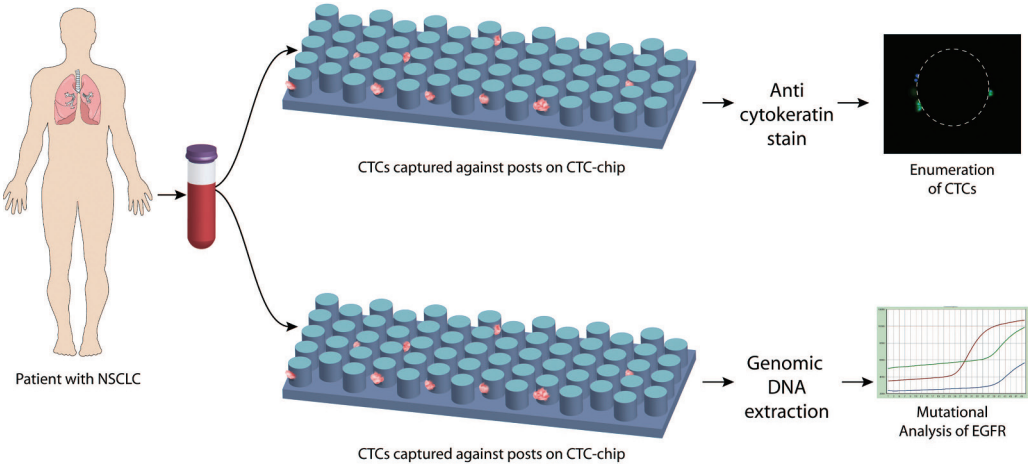

FIGURE 1. Illustrative cartoon demonstrating a patient with non-small cell lung cancer (NSCLC) donating a tube of peripheral blood which is then processed in the circulating tumor cell (CTC)-chip immediately, without any required preprocessing. CTCs are captured against the sides of the anti-Ep-CAM-coated posts (epithelial cell adhesion molecule), and then can be stained with fluorescently-labeled markers for enumeration or undergo genomic DNA extraction for epidermal growth factor receptor mutation or other molecular analysis.

antibodies could potentially be used to functionalize the microposts, resulting in the capacity to detect a wide variety of types of CTCs.

We piloted the CTC-chip in 116 blood samples from 68 patients with epithelial cancer diagnoses, including 55 blood samples from patients with advanced non-small cell lung cancer (NSCLC).[7] CTCs were isolated from all of the NSCLC samples (mean 155 cells/ml, standard deviation ±236). Similar results were also obtained among patients with other tumor types, including colorectal, esophageal, breast, and prostate cancer. Nine patients, including three with advanced NSCLC, were followed with serial blood samples for CTC analysis over the course of first or second-line chemotherapy treatment and a significant correlation was observed between the change in the quantity of CTCs captured and the response to treatment as measured by standard CT scans using RECIST criteria, Pearson's correlation coefficient = 0.68 ($p = 0.03$).[7]

These findings are remarkable and distinguishable from results obtained with other techniques in several ways, including the high number of CTCs isolated per blood sample, the ability to capture CTCs from essentially all patients tested, and the capacity to dynamically monitor CTC quantity in a meaningful way in real time. By way of comparison, other investigators have studied the use of the immunomagnetic bead-based CTC assay currently available for clinical use (CellSearch, Veridex LLC) on 168 blood samples from 99 patients with advanced lung cancer; and detected CTCs in 34 (20%) of the samples.[10] Only 10 (6%) of the lung cancer samples had >6 CTCs/ml captured, the rest had lesser numbers of cells identified. Given the low sensitivity and low yield of this method, we believe it may not prove as suitable for monitoring response to treatment as the CTC-chip platform, and studies are ongoing looking at the monitoring capabilities of the CTC-chip in many cancer settings.

In addition to high sensitivity, an important advantage of the CTC-chip is the ability to isolate cells while maintaining their viability (98% of captured cells are viable[7]). In contrast, the multiple-step process involved in the CellSearch system renders isolated CTCs nonviable[10] and therefore not usable for potential future functional analyses. We believe that the high capture rates and viable condition of the cells isolated via CTC-chip make it an ideal tool for molecular diagnostics.

In most types of cancer, cutting-edge, personalized clinical care is becoming more dependent upon accurate molecular diagnostic information. NSCLC is one of the prime examples of this trend because somatic mutations in the epidermal growth factor receptor (EGFR) gene are known to correlate with increased response and survival after treatment with tyrosine kinase inhibitors specific to the receptor.[11–14] Several recent studies confirm the benefit of screening appropriate patients for *EGFR* mutations at the time of diagnosis with advanced disease and making first-line therapy decisions based on the results.[14–17] The most notable of these is the IPASS study, a large randomized trial that demonstrated that using this strategy in never or low-smoking patients with adenocarcinoma yields a superior outcome. When patients with *EGFR* mutations were treated with first-line gefitinib, the hazard ratio for progression-free survival was 0.48 (95% confidence interval [CI] 0.36–0.68; $p < 0.0001$) compared with carboplatin and paclitaxel chemotherapy, and conversely when wild-type *EGFR* patients were treated with first-line gefitinib, the hazard ratio for progression-free survival was 2.85 (95% CI 2.05–3.98; $p < 0.0001$) compared with chemotherapy. This suggests that specific genotype is a more accurate predictor of benefit from anti-EGFR therapy than clinical phenotype surrogates, and should be a crucial component of first-line decision-making for a subset of NSCLC patients.[14] However, a practical limitation to this line of work has been the feasibility of obtaining sufficient tumor tissue to perform the genotype analyses in a time-frame that is clinically useful. Furthermore, the development of acquired resistance to EGFR tyrosine kinase inhibitors therapy is a

clinical issue about which we have some understanding of the molecular basis (for example T790M *EGFR* mutations and *MET* amplification), but additional research could be greatly enhanced by removing the barrier of lack of repeated access to tumor tissue.[18–22]

The CTC-chip may provide a solution to this problem. In a group of NSCLC patients known to harbor *EGFR* mutations, we compared direct sequencing of tumor tissue biopsies for *EGFR* mutations with an allele-specific *EGFR* mutation analysis of DNA from captured CTCs.[8] We were able to identify the expected *EGFR* mutation from CTCs in 92% of cases. In addition, in several patients followed serially over the course of their therapy with gefitinib, we not only documented the expected changes in CTC number correlating to clinical and radiographic responses, but were able to document the emergence of the T790M resistance mutation heralding clinical resistance, suggesting that CTC analysis could indeed be used as a pseudo repeat biopsy to look at changing genotypes in response to targeted therapies. The ability to perform noninvasive serial sampling of tumor genotypes represents a sea-change in translational research in NSCLC.

Although there is much work yet to be done to optimize the uses of the CTC-chip, its ultimate implications for NSCLC clinical management and research applications are vast. Pilot results already show promise in monitoring response to treatment in patients with advanced disease, which could be translated to both standard clinical management and clinical trials research. Given the controversy over the utility and cost of radiographic screening for lung cancer, a blood-based assay with high sensitivity like the CTC-chip is immensely appealing as a screening tool for high-risk populations like smokers.[23] The viability of the captured CTCs makes their potential use in basic biologic research nearly unlimited, with likely implications for understanding the process of metastasis. And perhaps most timely, the proven ability to analyze *EGFR* mutations by a peripheral blood sample via the CTC-chip has crucial implications for the field of targeted therapy and translational research.

In summary, the CTC-chip is a landmark technology that allows for isolation of large numbers of rare CTCs from the peripheral blood of cancer patients. The unique microfluidic platform holds promise for lung cancer research and clinical management, and in the near future may be used for early detection, monitoring of disease, and genotyping of tumors.

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